# Biotypic Diversity in Greenbug (Hemiptera: Aphididae): Characterizing New Virulence and Host Associations

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**ABSTRACT** Biotypic diversity of the greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), was assessed among populations collected from cultivated wheat, *Triticum aestivum* L., and sorghum, *Sorghum bicolor* (L.) Moench, and their associated noncultivated grass hosts. Greenbugs were collected during May through August 2002 from 30 counties of Kansas, Nebraska, Oklahoma, and Texas. Discounting the presumptive biotype A, five of the remaining nine letter-designated greenbug biotypes were collected; however, biotypes C, F, J, and K were not detected. Biotypes E and I exhibited the greatest host range and were the only biotypes collected in all four states. Sixteen greenbug clones, collected from eight plant species, exhibited unique biotype profiles. Eleven were collected from noncultivated grasses, three from wheat, and two from sorghum. The most virulent biotypes were collected from noncultivated hosts. The great degree of biotypic diversity among noncultivated grasses supports the contention that the greenbug species complex is composed of host-adapted races that diverged on grass species independently of, and well before, the advent of modern agriculture.

**KEY WORDS** biotype, plant resistance, *Schizaphis graminum*, *Sorghum bicolor*, *Triticum aestivum*

Biotypic variation among populations of greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), has been a driving force behind breeding programs for wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; and sorghum, *Sorghum bicolor* (L.) Moench since the designation of the first biotype by Wood in 1961 (Wood 1961). The first new greenbug strain was virulent to greenbug-resistant DS 28A wheat and was designated biotype B, with the presumption that all other greenbugs were avirulent, thus constituting biotype A. Wood also compared morphological and biological traits of the two biotypes and concluded that the damage response exhibited by the plant was the only reliable method to distinguish the newly discovered greenbug biotype. Conceptually, Wood adopted a classification scheme to describe greenbug biotypes from one that was devised by Painter et al. (1931) for characterizing virulent populations of Hessian fly, *Mayetiola destructor* (Say). Painter generally defined a biotype as a morphologically indistinct "subspecific strain" of an insect species that displayed unique responses to, or effects on, a genetically stable resistant host (Painter 1951). Since the identification of biotype B, eight additional biotypes have been differentiated based on their ability to damage resistant plants (Table 1). Numerous studies have been conducted to further characterize the interactions between biotypes and resistant and susceptible plants; however, the assessment of damage (virulence) to a specific set of resistant plants is the only criterion used to identify a greenbug biotype. Consequently, the genetic basis for identification of greenbug biotypes is plant based and not insect derived. Moreover, because the basis for distinguishing between biotypes of greenbugs is based on the response of a genotype of plant, the genetic composition of a greenbug biotype is further obscured by each biotype being a phenotypic expression of an indefinite number of genotypes (Puterka and Peters 1990).

Genetic data from inheritance of virulence studies (Puterka and Peters 1989, Ullah and Peters 1996) demonstrated that sexual reproduction is probably the primary mechanism responsible for the generation of new biotypes of greenbug and diversity among clones (Shufran et al. 1997). Substantial clonal diversity within greenbug biotypes further indicated that biotypes do not arise from single clones (Shufran et al. 1992). Although there is evidence for somatic mutations in clonal aphids (Lushai et al. 1998), attempts to increase aphid fitness to overcome plant resistance, i.e., to induce development of new biotypes, by continuous rearing of parthenogenic intraclonal aphids on resistant plants have failed (Starks and Schuster 1976, Di Pietro and Caillaud 1998).

Why greenbug biotypes have developed is a much more contentious question. Theoretically, it is widely held that selective pressures exerted by resistant crop

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**Table 1. Recognized greenbug biotypes, their resistance connotation, and reference**

| Biotype | Resistance source overcome | Reference |
|---|---|---|
| A | None (presumed biotype) | Wood (1961) |
| B | Wheat (*gb1*) | Wood (1961) |
| C | None (new to sorghum) | Harvey and Hackerott (1969) |
| E | Wheat (*Gb2*) | Porter et al. (1982) |
| F | None (damaged bluegrass) | Kindler and Spomer (1986) |
| G | Wheat (*gb1,Gb2,3,4,5*) | Puterka et al. (1988) |
| H | Wheat (*gb1,Gb2,4,5,6*), and Barley (*Rsg1a, Rsg2b*) | Puterka et al. (1988) |
| I | Sorghum (biotype E resistant) | Harvey et al. (1991) |
| J | None (avirulent to all resistance) | Beregovoy and Peters (1995) |
| K | Sorghum (biotype I resistant) | Harvey et al. (1997) |

cultivars led to the proliferation of biotypes. However, Porter et al. (1997) reviewed the chronology of deployment of resistant plants and the development of new greenbug biotypes and found that there was no apparent correspondence between the two. More recently, genetic studies have indicated that host-adapted races occur within the greenbug species complex (Shufran et al. 2000, Anstead et al. 2002). These races probably evolved on noncultivated grass hosts and have secondarily invaded cereal crops. On a limited scale, noncultivated grass hosts have been shown to play a role in generating and maintaining diversity of greenbug biotypes (Anstead et al. 2003). Greenbugs have a sizable host range that includes 70 poaceous species (Michels 1986); however, little is known of the occurrence or extent of the biotypic diversity of greenbugs on noncultivated hosts. This information is crucial to understanding the relationship between host-adapted races, biotype development, and plant resistance. The objective of this study was to assess, on a regional scale, the relative amount of biotypic diversity (new virulence genes) among populations of greenbugs collected from cultivated wheat and sorghum and their associated noncultivated grass hosts.

## Materials and Methods

**Collections in the Field.** Greenbugs were collected from May through August 2002 from 30 counties of Texas, Oklahoma, Kansas, and Nebraska (Fig. 1). Al least two fields, separated by a minimum distance of 3.2 km, in each county were sampled using a Stihl model 85 leaf blower-vacuum (Stihl Incorporated, VA Beach, Virginia) customized to function as a D-vac system through attachment of a fine mesh collection bag onto the vacuum tube (10 cm in diameter). This system made it possible to sample specific plants precisely and facilitated the sampling of a large number of plants in a short time. Samples were collected from cultivated wheat and sorghum as well as the noncultivated grasses along the field margins. The noncultivated grasses were sampled discretely within 1–5 m from the margins of cultivated fields, and greenbugs collected from each grass species were kept separately. Greenbugs were transferred from the collection bag to 'Schuyler' barley seedlings that were caged with ventilated clear-plastic cylinders to prevent cross-sample contamination. Subsequent clonal colonies for evaluation of biotype were established at the laboratory by selecting a single, apterous greenbug from each noncultivated grass sample. Three test colonies were derived from each greenbug sample collected from wheat or sorghum; two clonal colonies were established by selecting two individual, apterous greenbugs from the sample, and the third test colony was composed of the remainder of the original sample. The individual aphids selected for establishing the clonal colonies were carefully selected from separate aggregations on the host plant, and selection preference was given to aphids exhibiting any phenotypic difference (i.e., color). All aphids were checked daily for the presence of parasitization readily detected during early formation of mummies. All mummified aphids were removed before the parasitic wasp emerged. Test colonies were reared on Schuyler barley grown in caged pots and maintained in environmental chambers with a photoperiod of 16:8 (L:D) h at 20 and 18°C, respectively.

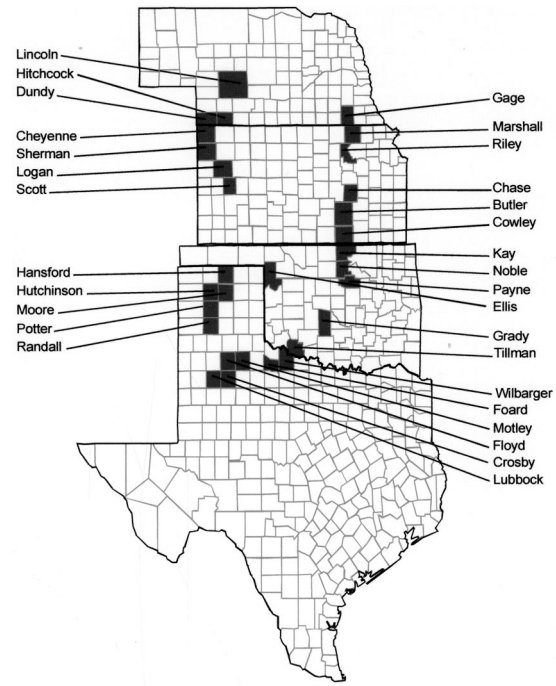


**Fig. 1.** Counties where greenbugs were sampled.

**Table 2. Greenbug-resistant sources used for determining biotypes**

| Plant resistance source | Resistance gene designation | Expressed biotype resistance |
|---|---|---|
| Wheat | | |
| Custer | Susceptible check | — |
| DS 28A | *gb1* | A, F, J |
| Amigo | *Gb2* | B, C, J |
| Largo | *Gb3* | C, E, H, I, J, K |
| CI 17959 | *Gb4* | C, E, I, J, K |
| CI 17882 | *Gb5* | C, E, I, J, K |
| GRS 1201 | *Gb6* | B, C, E, G, I, J, K |
| Rye | | |
| Elbon | Susceptible check | — |
| Insave | *Gb2*, *Gb6* | B, C, E, G, I, J, K |
| Barley | | |
| Wintermalt | Susceptible check | G |
| Post 90 | *Rsg1a* | B, C, E, F, G, I, J, K |
| PI 426756 | *Rsg2b* | B, C, E, F, G, I, J, K |
| Sorghum | | |
| TX 7000 | Susceptible check | — |
| TX 2737 | — | C |
| TX 2783 | — | C, E |
| PI 550607 | — | B, C, E, G, H, I |

Data are from Wood (1961), Harvey and Hackerott (1969), Teetes et al. (1974), Johnson et al. (1982), Porter et al. (1982), Peterson et al. (1984), Kindler and Spomer (1986), Puterka et al. (1988), Harvey et al. (1991), Andrews et al. (1993), Beregovoy and Peters (1995), Harvey et al. (1997), and Peters et al. (1997).

**Table 3. Biotype composition of greenbugs collected from the field in Kansas, Nebraska, Oklahoma, and Texas**

| State and County | Greenbug biotype | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | B | C | E | F | G | H | I | K | New |
| Kansas | | | | | | | | | |
| Butler | | | X | | | | X | | |
| Chase | | | X | | | | X | | |
| Cheyenne | | | X | | X | | | | |
| Cowley | | | X | | | | X | | |
| Logan | | | X | | X | | X | | X |
| Marshall | | | X | | | | X | | |
| Riley | | | X | | | | X | | X |
| Scott | | | | | X | | X | | X |
| Sherman | X | | X | | X | | X | | |
| Nebraska | | | | | | | | | |
| Dundy | | | X | | | | | | |
| Gage | | | X | | | | X | | |
| Hitchcock | | | X | | | | | | |
| Lincoln | | | X | | | | X | | |
| Oklahoma | | | | | | | | | |
| Ellis | | | X | | | | X | | |
| Grady | | | X | | | | X | | |
| Kay | | | X | | | | X | | |
| Noble | | | X | | | | X | | |
| Payne | | | X | | | | X | | |
| Tillman | X | | X | | X | X | X | | X |
| Texas | | | | | | | | | |
| Crosby | | | X | | | | X | | |
| Floyd | | | X | | | | X | | |
| Foard | | | X | | | | X | | |
| Hansford | | | X | | | | X | | X |
| Hutchinson | | | X | | | | X | | X |
| Lubbock | | | X | | | | X | | |
| Moore | | | X | | | | X | | X |
| Motley | | | X | | | | X | | |
| Potter | | | X | | | | X | | X |
| Randall | | | X | | | | X | | X |
| Wilbarger | | | X | | X | X | X | | X |

**Determination of Biotype.** A general overview of the procedures used for determination of biotypes was described by Starks and Burton (1977). The biotypic status of each test colony was determined using previously established plant differentials of barley; rye, *Secale cereale* L.; sorghum; and wheat (Table 2). Seeds of each plant genotype were planted in separate rows, at a rate of 10 seeds per 15-cm row, with four replications, in flats on greenhouse benches. Genotypes of plants were randomly assigned to rows. Barley, rye, and wheat plants were tested separately from sorghum. Before testing, each greenbug test colony population was increased to ensure adequate abundance of aphids for testing. Immediately after planting, the flats containing the test plants were caged to ensure that secondary aphids would not contaminate the plants. The caged plants were infested at the two-leaf stage by cutting and placing infested leaves next to each row of test plants. The tests of barley, rye, and wheat plants were done under supplemental artificial light with a photoperiod of 16:8 (L:D) h and 22 ± 5°C in a greenhouse. The conditions for the sorghum tests were the same except the temperature was maintained at 28 ± 5°C. Once the susceptible control plants ('Custer' wheat, 'Elbon' rye, and 'Wintermalt' barley, or TX 7000 sorghum) were killed (usually within 7–14 d), the test was terminated, and the plants were scored as alive (resistant) or dead (susceptible). Under these test conditions, plant responses were discrete and easily scored. When a new biotype was discovered, the evaluation process was repeated to confirm the results. A greenbug isolate was considered a new biotype when its plant response profile was unique. New biotypes were not designated using the customary sequential alphabetic letters; instead, they were denoted with regard to the state from which they were collected and numbered sequentially. After each test, vouchers of the aphids were collected and deposited at the Cereal Insect Genetic Resource Library, USDA–ARS, Plant Science Research Laboratory, Stillwater, OK.

## Results

Greenbugs were collected from 112 wheat and sorghum fields, and the noncultivated grasses along their margins, from 30 counties of four states. The biotype composition of the samples from the field is listed in Table 3. Discounting the presumptive biotype A, five of the remaining nine letter designated biotypes were collected; only C, F, J, and K were not detected. Biotypes E and I were the most ubiquitous and the only biotypes collected in all four states.

Greenbugs collected from Nebraska had the least biotypic diversity among greenbug populations, and only biotypes E and I were found. Extensive sampling of noncultivated grasses yielded only those biotypes present in the neighboring wheat or sorghum field. This lack of diversity is consistent with previous reports that identified biotypes E and I to be dominant in Nebraska (Kindler et al. 1984, Bowling et al. 1994).

Biotypes E and I were also dominant in Kansas, each occurring in all but one of the counties sampled. In contrast to Nebraska, samples from Kansas had a much greater biotypic diversity among the documented biotypes. Biotype G was present in all of the western counties sampled (Fig. 1) and was collected from both wheat and noncultivated grasses. Biotype B also was found in western Kansas and was collected from wheat and jointed goatgrass, *Aegilops cylindrica* Host. In addition, three new biotypes, denoted KS1, KS2, and KS3, and a population previously identified as the New York (NY) isolate (Shufran et al. 2000) were collected.

In Oklahoma, biotypes E and I were present in all counties sampled. Although biotypic diversity was lacking in most counties, biotypes B, G, and H, and three new biotypes were found in Tillman County, Oklahoma. Biotype B was collected from sorghum and barnyardgrass, *Echinochloa crusgalli* (L.) Beauv., biotype G from wheat, and biotype H from jointed goatgrass. The three new biotypes collected were denoted OK1, OK2, and OK3. The biotype composition observed in Oklahoma is generally consistent with the greenbug surveys previously reported by Kerns et al. (1987) and Peters et al. (1997), but we did not find biotype C.

Biotypes E and I were the biotypes most commonly found in Texas, both occurring in all counties sampled. Biotype G was collected from wheat, and biotype H from jointed goatgrass and intermediate wheatgrass, *Agropyron intermedium* (Host.) Beauv., in Wilbarger County, Texas. As in Oklahoma, biotype C was not found in Texas. This is in contrast to the survey report by Bush et al. (1987) who found biotype C to make up ≈10% of the greenbugs sampled. Ten new biotypes, denoted TX1 through TX10, were found in six of the 11 counties sampled in Texas.

The host species from which the different greenbug biotypes were collected are shown in Table 4. As would be expected from their pervasive distributions, biotypes E and I exhibited the greatest ranges of host species and were collected from 13 and seven plant species, respectively. Biotypes B, E, and I were the only biotypes collected from both wheat and sorghum, and both biotypes E and I were found on maize, *Zea mays* L. Jointed goatgrass, wheatgrasses (*Agropyron* spp.), and Johnsongrass, *Sorghum halepense* (L.) Pers., seem to be the most important grasses for harboring diverse populations of greenbug biotypes associated with cultivation of sorghum and wheat.

Sixteen greenbug clones, collected from eight plant species, had biotypic profiles different from all previously reported biotypes (Table 5). Six of the clones were collected from Johnsongrass, three from wheat, two from sorghum, and one each from cheatgrass, *Bromus secalinus* L.; downy brome, *Bromus tectorum* L.; jointed goatgrass; western wheatgrass, *Agropyron smithii* Rydb.; and Canada wildrye, *Elymus canadensis* L. Although wheat and sorghum harbored several biotypes, including five new greenbug clones, much greater biotypic diversity was found among the populations of greenbugs collected from noncultivated grasses.

**Table 4. Biotypes of greenbugs collected from maize, sorghum, wheat, and noncultivated grasses**

| Host | Greenbug biotype | | | | | |
|---|---|---|---|---|---|---|
| | B | E | G | H | I | New (*n*) |
| *A. cylindrica* Jointed goatgrass | X | X | | | | X(1) |
| *A. smithii* Western wheatgrass | | X | X | | | X(1) |
| *A. intermedium* Intermediate wheatgrass | | | X | | X | |
| *A. cristatum* (L.) Gaertn. Crested wheatgrass | | | X | | | |
| *Andropogon gerardii* Big bluestem | | X | | | X | |
| *Avena sativa* L. Oat | | X | | | | |
| *B. secalinus* Cheatgrass | | X | | | | X(1) |
| *B. tectorum* Downy brome | | X | | | | X(1) |
| *B. inermis* Smooth brome | | X | | | X | |
| *E. crusgalli* Barnyardgrass | X | X | | | | |
| *E. canadensis* Canada wildrye | | X | | | | X(1) |
| *S. bicolor* Sorghum | X | X | | | X | X(2) |
| *S. halepense* Johnsongrass | | X | | | X | X(6) |
| *T. aestivum* Wheat | X | X | X | | X | X(4) |
| *Z. mays* Maize | | X | | | X | |

The biotypic profiles for all documented biotypes and new greenbug clones are shown in Fig. 2. Thirteen of the 16 new greenbug clones collected during this study were unique. TX4 and TX5, collected on Johnsongrass and wheat, respectively, shared the same biotypic profile. Similarly, KS2 (Canada wildrye) and

**Table 5. Designation, host, and county record of new greenbug biotypes**

| Population designation | Host | Associated cropping system | County |
|---|---|---|---|
| Kansas | | | |
| KS 1 | *A. smithii* | Wheat/sorghum | Logan |
| KS 2 | *E. canadensis* | Wheat/sorghum | Scott |
| KS 3 | *T. aestivum* | Wheat/fallow | Riley |
| Oklahoma | | | |
| OK 1 | *S. halepense* | Wheat/sorghum | Tillman |
| OK 2 | *S. halepense* | Wheat/sorghum | Tillman |
| OK 3 | *S. bicolor* | Wheat/sorghum | Tillman |
| Texas | | | |
| TX 1 | *S. halepense* | Wheat/sorghum | Potter |
| TX 2 | *T. aestivum* | Wheat/sorghum | Potter |
| TX 3 | *S. bicolor* | Wheat/sorghum | Potter |
| TX 4 | *S. halepense* | Wheat/sorghum | Potter |
| TX 5 | *T. aestivum* | Wheat/sorghum | Randall |
| TX 6 | *A. cylindrica* | Wheat/sorghum | Moore |
| TX 7 | *B. secalinus* | Wheat/fallow | Hutchinson |
| TX 8 | *S. halepense* | Wheat/fallow | Hansford |
| TX 9 | *S. halepense* | Wheat/sorghum | Wilbarger |
| TX 10 | *B. tectorum* | Wheat/sorghum | Moore |

| Cereal selection (R-gene) | A | B | C | E | F | G | H | I | J | K | C WR | WWG | NY | KS 1 | TX 1 | TX 2 | TX 3 | TX 4 | TX 5 | KS 2 | TX 6 | TX 7 | TX 8 | KS 3 | OK 1 | OK 2 | TX 9 | TX 10 | OK 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Custer | S | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | R | S |
| DS 28A (gb1) | R | S | S | S | R | S | S | S | R | S | S | S | R | R | R | R | R | R | R | R | R | R | S | S | S | S | S | R | S |
| Amigo (Gb2) | | R | R | S | S | S | S | S | R | S | R | S | R | R | S | S | S | S | S | S | S | S | S | S | R | S | S | R | S |
| CI 17882 (Gb5) | | S | R | R | S | S | S | R | R | R | S | R | S | S | S | S | S | R | R | R | R | R | S | R | S | S | S | R | S |
| CI 17959 (Gb4) | | S | R | R | S | S | S | R | R | R | S | R | S | S | S | S | S | R | R | R | R | R | S | R | S | S | S | R | R |
| Largo (Gb3) | | S | R | R | S | S | R | R | R | R | S | S | S | S | S | S | S | R | R | R | R | R | S | R | S | S | S | R | R |
| GRS 1201 (Gb6) | | R | R | R | S | R | S | R | R | R | S | R | S | S | R | R | R | R | R | R | R | R | R | R | R | S | S | R | S |
| Elbon | | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | R | S |
| Insave (Gb2, Gb6) | | R | R | R | S | R | S | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | S | S | R | S |
| Wintermalt | | S | S | S | S | R | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| Post 90 (Rsg1a) | | R | R | R | R | R | S | R | R | R | R | R | R | R | S | S | S | S | S | S | S | S | S | R | R | S | S | S | S |
| PI 426756 (Rsg2b) | | R | R | R | R | R | S | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | S | S | S | S | S | S | S |
| TX 7000 | | --- | S | S | --- | S | --- | S | --- | S | S | --- | S | R | S | S | S | S | S | S | S | S | S | S | S | R | R | R | S |
| TX 2737 | | --- | R | S | --- | S | --- | S | --- | S | R | --- | S | R | S | R | S | S | S | S | S | S | R | S | S | S | S | R | S |
| TX 2783 | | S | R | R | S | S | --- | S | --- | S | S | --- | S | S | S | S | S | S | S | R | R | S | S | R | S | S | S | R | S |
| PI 550607 | | R | R | R | S | R | R | R | --- | S | R | --- | R | R | S | R | R | S | S | R | R | R | R | R | R | R | R | R | R |

**Fig. 2.** Plant reactions to known greenbug biotypes and unique greenbug isolates collected from Kansas (KS), Oklahoma (OK), and Texas (TX). R and S indicate resistant and susceptible reactions, respectively; —indicates data not available. CWR, Canada wildrye isolate; NY = New York isolate; WWG, western wheatgrass isolate.

TX6 (jointed goatgrass) as well as OK2 (Johnsongrass) and TX9 (Johnsongrass) had the same biotypic profiles. DS 28A (*gb1*), long thought to be of limited value for resistance to greenbug, was resistant to eight of the 13 new biotypes. In contrast, Post 90 (*Rsg1a*), which was very resistant to 11 of the 12 previously reported biotypes, was susceptible to 10 of the 13 new biotypes. Overall, GRS 1201 (*Gb6*) and 'Insave' (*Gb2, Gb6*) provided the widest range of resistance to the new biotypes. Several of the new biotypes had the same virulence pattern as biotypes E, I, or K on the resistant sorghums. Consequently, they may have gone undetected in previous biotype surveys where greenbugs were evaluated solely on resistant sorghums.

## Discussion

Greenbug biotypes have been defined by their virulence relationship to a select group of plant genotypes. As more resistant genotypes of crop plants were developed, new biotypes of greenbugs were discovered. A gene-for-gene model was assumed, whereby newly developed resistant cultivars exerted selective pressure on the current predominant biotype, which in turn led to the development of a new virulent biotype. Porter et al. (1997) challenged this scenario and exposed several potential flaws in the application of this model to development of greenbug biotypes. Porter et al. (1997) postulated that biotypic diversity (variability for virulence) existed naturally within greenbug populations and occurred long before the deployment of resistance. Subsequent phylogenetic studies, based on mitochondrial DNA divergence (COI haplotypes), have shown that biotypes of greenbugs identified solely from response by plant differentials are not discrete populations and the classification of biotype has neither an evolutionary nor taxonomic basis (Shufran et al. 2000, Anstead et al. 2002). Instead, it was concluded that the greenbug species complex is made up of host-adapted races that have diverged on noncultivated grass species well before the advent of modern agriculture, and biotypes are comprised of genetically diverse individuals among different host races that merely share similar virulence genes (Anstead et al. 2002). In the current study we assessed the composition of greenbug biotypes on wheat, sorghum, and noncultivated grasses over a large area and found that overall biotypic diversity was much greater among greenbugs collected from noncultivated grasses, which would be expected if greenbugs diverged on grasses. The most virulent biotypes were not present on wheat or sorghum, but they were collected from noncultivated grass hosts, thus providing further evidence that these hosts, not cultivated crops, are key to the development and maintenance of genetic diversity for virulence in greenbugs. Consequently, the putative relationship between greenbug virulence and reproductive fitness, which is assumed by the gene-for-gene model, is dubious because the more virulent biotypes were rarely found infesting cultivated crops (Fig. 2).

Thirteen new biotypes were identified, which is remarkable considering that only nine biotypes have been described previously, only 16 genotypes of plants (12 resistant and four susceptible) were used to evaluate virulence, and collections of greenbugs were limited to noncultivated grasses along the margins of cultivated fields in a single growing season. Undoubtedly, this collection represents only a fraction of extant biotypes. Six of the new biotypes were collected from Johnsongrass, which confirms its importance as a reservoir for biotype diversity. That the new biotypes were not widely distributed (i.e., only three

occurred in more than one county), and were not collected on more than one noncultivated host species, suggests that some greenbug populations do not widely disperse. The ubiquitous distribution of biotypes E and I may result from their wide noncultivated host range (i.e., comprised of several host-adapted races) and their ability to exploit wheat and sorghum. They are currently the only reported biotypes that cause widespread economic damage to crops, yet they constitute a very small segment of the overall biotype community.

In summary, our findings support the contention that the greenbug species complex is composed of host-adapted races that diverged on noncultivated grasses, and greenbug biotypes that occur on cultivated wheat and sorghum are small subsets of these host-adapted races. Plant resistance to greenbugs will continue to be an important strategy in pest management; however, plant breeders should consider local host races, not just the dominant biotypes, when searching for sources of resistance in plant improvement programs.

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